LXIX. AN IMMEDIATE ACID CHANGE IN SHED BLOOD.

BY ROBERT EMLYN HAVARD¹
AND PHYLLIS TOOKEY KERRIDGE².

From the Medical Unit Laboratory, London Hospital, and the Department of Physiology and Biochemistry, University College, London.

(Received June 11th, 1929.)

INTRODUCTION.

THE p_H of blood was measured at body temperature by the glass electrode method about 2 minutes after the withdrawal of the blood from the blood-vessel. The p_H was observed to decrease by about 0.05 a few minutes later. This acid change seemed to be very important, as it meant that later determinations do not represent the p_H of the blood in the body. A typical p_H -time curve is shown in Fig. 1, curve 1. This p_H change was investigated further.

TECHNIQUE.

(a) *Collection of blood.*

Human blood was mainly used in this research, the total number of individuals from whom one or more blood samples were taken being 21. No special attempt was made to control the physiological conditions which might affect the c_H of the blood, but the subject sat still for 5–10 minutes before the blood-vessel was punctured. Stasis was generally avoided, but in the later experiments it was used when convenient.

The skin was cleaned with an antiseptic, the vessel punctured with a sterile needle, and the blood was drawn into a syringe. Contact of the blood with air was avoided by filling the dead space of the syringe and needle with liquid paraffin. Change of temperature during this manipulation was reduced to a minimum. This could be done by using a small all-glass syringe, previously warmed to 38°. When a syringe with metal plunger and cap was used it was necessary to warm it to about 45°, and to lag it with cloth, as otherwise the conductivity of the metal caused it to cool quickly.

In the experiments in which chemical substances in solution were added to the blood, these were measured into the syringe before the puncture. The

¹ Beaney Research Student, Guy's Hospital.

² Working for the Medical Research Council.

presence of a glass bead proved essential to ensure complete mixing. No liquid paraffin was used in these cases (except as a piston lubricant). The needle was inserted into the vessel, and blood allowed to flow through it before the syringe was attached. In this way air was removed from the needle and there was no danger of traces of the chemical entering into the circulation.

(b) *Measurement of p_H .*

The hydrogen ion concentration of the blood was measured by the glass electrode method [Kerridge, 1926]. The blood was transferred immediately from the warm syringe to a standardised electrode, which was at body temperature, and the first c_H determination completed between one or two minutes after the blood left the vessel.

Two completely independent glass electrode apparatus were used, so that simultaneous p_H -time curves could be obtained from samples of blood either at two different temperatures, or with and without added substances. The calomel and glass electrodes of each apparatus were enclosed in a large air-bath with automatic temperature regulation and an efficient fan. The air-baths were lined with metal, which was connected to earth. When the door of an air-bath was opened, as for the introduction of the sample of blood, the fan was stopped, and an accessory heating lamp, situated near the door, was turned on. It was very important that this procedure should be completed as quickly as possible, and the fan turned on again as soon as the door was shut. The temperature remained constant within 0.5° under these circumstances. The glass electrodes were all made of Wood's glass of the formula recommended by Hughes [1928]. They were standardised daily against a solution of $M/20$ potassium hydrogen phthalate, the p_H of which was taken to be 3.97° , both at room temperature and at 37.5° . The standardisations were carried out at the same temperature as the subsequent experiment. From time to time, the electrodes were checked by determining the p_H of a dilute phosphate buffer solution, of about p_H 7.3, which was also estimated by the quinhydrone electrode method. The calomel electrodes contained $3.5 M$ KCl, and the potassium phosphate buffer used on one side of the glass membrane was dilute (approx. $M/20$). Under these circumstances the liquid-liquid junction potentials can be neglected, but evidence was obtained which showed that this is by no means the case when the calomel electrodes contain $M/10$ KCl and the phosphate buffer solution is concentrated. Special care was taken with the ground caps of the calomel electrodes, as KCl may diffuse into the electrode during long experiments when these fit badly.

The Lindemann electrometers were adjusted so that a deflection caused by 0.25 mv. (corresponding to $0.004 p_H$) could be detected. By the kind permission and courteous cooperation of the Cambridge Instrument Co., both potentiometers were calibrated at their works for zero error and uniformity of slide wire against one of their standard potentiometers. The potentiometers

were standardised before each experiment with a Weston cadmium cell. The error in the potentiometer readings is estimated at 0.5 %. Control experiments in which the p_H of the same liquid was measured in the two apparatus agreed within 0.02 p_H . This difference gives the order of the combined instrumental and manipulative errors. The control experiments were done both on buffer solutions, whose p_H was checked by the quinhydrone electrode method, and on blood. Thus it may be said that the absolute p_H measurements were accurate to 0.02 p_H , but that differences of 0.005 p_H on any one sample were significant.

EXPERIMENTS.

(i) *Effect of sodium fluoride.*

Evans [1922] has described changes in blood shortly after shedding, which were stopped by sodium fluoride. We found that addition of 0.02 % sodium fluoride to the blood caused an almost complete disappearance of the change in p_H corresponding to the part of the p_H -time curve denoted at *DE* in Fig. 1. The inhibition was complete in greater concentrations, and we used 0.06 % in subsequent experiments in which we wished to eliminate this p_H change (cf. Fig. 1, curve 2).

Fig. 1. p_H -time curves of shed blood.

Curve 1. Venous blood (human).

Curve 2. Venous blood (human) + 0.06 % sodium fluoride.

Curve 3. Arterial blood (cat under chloroform) + 0.04 % potassium oxalate + 0.06 % sodium fluoride: kept at 0° until 1 min. before experiment.

The abscissae represent time in minutes after the blood left the vessel in curves 1 and 2, and after the blood was put in the electrode in curve 3. The rectangles represent size of experimental errors.

AB corresponds to "latent period."

BC corresponds to "first acid change."

DE corresponds to acid change inhibited by sodium fluoride.

We propose to use in the remainder of this paper the term "first acid change" for the change in p_H corresponding to the part of the curve in Fig. 1 labelled *BC*. It is this change which has occupied most of our attention. The time before the beginning of the first acid change, labelled *AB*, will be referred to as the "latent period."

(ii) *The "first acid change."*

Particulars of p_{H} -time curves at 38° and 28° are given in Table I. Both the latent period and the duration were greater at the lower temperature, although they varied between rather wide limits. No figure has been given for the latent period of venous blood at 38° as in 12 out of 31 experiments the "first acid change" had apparently commenced before the first p_{H} reading was taken about 2 minutes after shedding; of the remainder, 10 latent periods were between 2 and 8 minutes, and the others had widely different values up to a maximum of 40 minutes.

A decrease in p_{H} of 0.05 corresponds to a 12.5 % increase in hydrogen ion concentration, and an average deviation from the mean of 0.015 p_{H} , to a 3.5 % variation. The variation is of the same order when only repeated determinations on the blood of a single individual are considered.

There were no significant differences between the results on arterial and on venous blood.

Table I.

Temperature	Venous blood		Venous blood + 0.04 % K oxal. + 0.06 % NaF		Arterial blood 38°
	38°	28°	38°	28°	
Latent period (min.):					
Mean	—	11	6	19	7.5
Maximum	40	13	14	30	17
Minimum	1.5	8	3	12	2.5
First acid change (p_{H}):					
Mean	0.055	0.05	0.04	0.05	0.05
Maximum	0.095	0.06	0.08	0.09	0.08
Minimum	0.02	0.03	0.015	0.03	0.025
Average deviation from mean	0.015	—	0.01	0.02	0.015
No. of experiments	31	3	9	7	7
Duration of first acid change (min.):					
Mean	9	5	10	18	5
Maximum	21	9	15	30	9
Minimum	2	2.5	4	2	2.5

At room temperature ($19.5^{\circ} \pm 1.5^{\circ}$) there was no significant change in p_{H} , greater than the experimental error, within about $1\frac{1}{2}$ hours after the blood was shed, but then an acid change took place very slowly, after which the p_{H} again became constant.

(iii) *Effect of anticoagulants.*

In the course of the experiments already described the blood clotted in the electrode. The following anticoagulants were added to the blood, without affecting the "first acid change":

Heparin 0.02 %, 0.04 % and 0.08 %; sodium fluoride 0.3 %; ammonium oxalate 0.045 %; potassium oxalate 0.04 %.

The substances, dissolved in isotonic saline, were added to the blood in such concentration as to give a 10 % dilution of the blood. Control experiments,

in which dilution was made with isotonic saline alone, showed no measurable change in the initial p_H of the blood or in the "first acid change."

Heparin (0.02, 0.04 or 0.08 %) and sodium fluoride (0.06 %) added to the blood together do not inhibit the acid change corresponding to the part of the curve in Fig. 1 labelled *DE*, which is inhibited by sodium fluoride alone. We have no explanation to offer.

Data referring to the "first acid change" in the presence of potassium oxalate (0.04 %) and sodium fluoride (0.06 %) are given in Table I, columns 3 and 4. It will be noted that the mean latent period is greater than in the case of blood alone, and that the mean size of the "first acid change" is less, although the difference in dimension is of questionable significance.

(iv) *Effect of potassium cyanide and of thymol.*

Potassium cyanide was added in $M/100$ concentration, without affecting the shape of the p_H -time curve. The cyanide made the initial p_H more alkaline, unless it was previously neutralised. Thymol ($M/500$) likewise had no effect.

(v) *p_H -time curves of plasma.*

The latent period of the "first acid change" of venous blood containing potassium oxalate (0.04 %) and sodium fluoride (0.06 %) had been found to have a mean value of 19 minutes at 28°. In another series of experiments the blood was centrifuged at 28° during this latent period, and the plasma was separated and placed in the electrode. In a typical experiment centrifuging was carried out between the fourth and eighth minutes, and the first p_H measurements were made at the tenth minute after the blood was shed. The plasma p_H -time curves showed no "first acid change." Corpuscles with adherent plasma showed typical p_H -time curves with a "first acid change." Plasma to which corpuscles were added to give one-quarter of the normal number showed curves with a typical "first acid change."

(vi) *Effect of laking the blood.*

p_H -time curves on laked blood were made from venous blood containing potassium oxalate (0.04 %), sodium fluoride (0.06 %) and saponin (1 %). These showed typical "first acid changes." The same result was obtained when laked blood was centrifuged at room temperature, before the experiment, to free the fluid from white cells.

(vii) *Miscellaneous experiments.*

Any possible unexplained electrical effects of the fibrin or of the corpuscles settling on to the glass membrane were ruled out as the cause of the "first acid change" by reversing the customary arrangement of buffer and blood in the electrode. The "first acid change" still occurred when the corpuscles dropped away from the membrane.

Two samples of blood with potassium oxalate and sodium fluoride kept at 38° for 0.5 hour, and for 1 hour respectively, before transference to the

glass electrode, showed no change of p_H with time. Their p_H agreed with the final p_H of another sample of blood which had been placed in the electrode as soon as possible after it was shed, and which had undergone a typical "first acid change" in the electrode. Hence in the first two cases the "first acid change" may be presumed to have taken place while the blood was still in the syringe, and to be quite independent of the electrode. Similarly blood containing heparin and hydroxyquinoline potassium sulphate (as an anti-septic) showed no change of p_H with time after 22.5 hours at 38°.

Estimations were made of the total carbon dioxide and of the oxygen contents of blood before and after the "first acid change". In these experiments a sample of blood was drawn into a syringe at 0°, which contained potassium oxalate and sodium fluoride. One portion was taken immediately for analysis. The remainder was warmed to 38° and kept at that temperature for 0.5 hour before an analysis was made. The method of analysis used was that of Harington and Van Slyke [1924]. No appreciable difference between the O_2 and total CO_2 contents of the two samples could be detected.

Samples of blood were taken in a similar way for lactic acid estimations. These were kindly made for us by Dr H. D. Kay by the method of Friedemann, Cotonio and Shaffer [1927], on the ether extract of a trichloroacetic acid filtrate of the blood. No significant difference between the results on the two samples was observed. However, it is possible that the "first acid change" might be occasioned in the first sample in the course of the precipitation with trichloroacetic acid, and it is therefore impossible to pronounce definitely that the acid which causes the shift in hydrogen ion concentration is not lactic acid.

DISCUSSION.

The results of the experiments described in the previous section may be summarised as follows. The "first acid change" is not associated with the clotting of blood; it is delayed by decrease of temperature; it is not affected by substances which inhibit oxidation phenomena (KCN), enzyme action (thymol), or glycolysis (NaF). It does not occur in plasma, but does occur when the red corpuscle wall is broken down and in the absence of leucocytes. No explanation has been found.

There are references in the literature to changes in p_H accompanying clotting. Kugelmass [1922] reported an alkaline change of 0.3 p_H , using a hydrogen electrode on a solution of diluted oxalated plasma. Hirsch [1924] found an acid change of 0.09 p_H in rabbit's plasma by a colorimetric method. Ross [1921] found no change in the p_H of blood on clotting. Our experiments confirm those of Ross, as the p_H -time curves of blood at 38° and 28° were unaffected by the addition of anticoagulants, and as in the experiments at room temperature the blood clotted in the electrode, while the p_H remained constant.

The existence of this unexplained "first acid change" signifies that p_H measurements of blood at 38° made more than about 4 minutes after the

blood is shed will be more acid than when it leaves the blood-vessel, by an average of 0.05 p_H . This appears to be due to an increase in acidity within the red corpuscle, as it occurs in centrifuged laked blood, but not in plasma. From the buffer curves of blood of Van Slyke, Hastings, and Neill [1922], it can be calculated that an increase in acidity of 0.05 p_H corresponds to an addition of 1.2 millimol of acid per litre of blood. Assuming that this p_H change occurs at constant CO_2 content, it must be accompanied by a disturbance of the $\frac{[H_2CO_3]}{[BHCO_3]}$ ratio in the blood. The corresponding changes in $[H_2CO_3]$ and $[BHCO_3]$ can be calculated by the Henderson-Hasselbalch equation. At 38°, assuming $pK = 6.17$ and constant, the change in $[H_2CO_3]$ is 11 %, in $[BHCO_3]$ 5 %. Thus, blood having initial pCO_2 of 48 mm., and combined CO_2 of 45.5 vols. %, would have 53 mm. and 43.3 vols. % respectively after the "first acid change." Thus, delayed determinations of pCO_2 and vCO_2 of blood will misrepresent the amounts at the time of shedding by this amount. At 28°, as the CO_2 tension is lower, the difference in pCO_2 comes within the experimental error of the methods available, thus thwarting an attempt to demonstrate independently the "first acid change" by measuring the CO_2 tension of blood during the latent period, and after the acid change had taken place. A "first acid change" has been also observed in the blood of cats, dogs and rabbits. Experiments on the behaviour of blood in pathological conditions are in progress.

PRECAUTIONS FOR MEASUREMENT OF p_H OF BLOOD.

There remains to be considered the best practical method of measuring the p_H of blood as it leaves the blood-vessel. It may be estimated at 38° within 1 or 2 minutes, with care taken to avoid cooling in transference from vessel to electrode. This, however, is not always convenient. Secondly, potassium oxalate (0.04 %) and sodium fluoride (0.06 %) may be added, the determination made less hurriedly and a correction of 0.05 p_H applied for the "first acid change." The average variation from the mean correction in normals is only $\pm 0.015 p_H$, but nevertheless the method is objectionable as we have no evidence as to the size of the correction, or as to its variation in different physiological conditions, or in pathological states. A third method is to measure the p_H at room temperature, when no acid change occurs up to 1.5–2 hours after the blood is shed, and to apply a temperature correction. We have found the mean p_H of venous blood to be 7.32 at 38°, 7.47 at 28°, and 7.60 at 19.5°. These correspond to a change in p_H of 0.015 per degree. Martin and Lepper [1926] give 0.011 p_H per degree. A correction of this magnitude introduces a considerable degree of uncertainty, especially as nothing is known of its variation in different physiological and pathological conditions of the blood. A fourth method is as follows.

The blood is rapidly cooled by drawing into an ice-cold syringe, and is kept in the syringe on ice until convenient. (Care must be taken that the

temperature is not very much below 0° , or the blood will freeze, and lake on thawing.) The blood is brought to room temperature immediately before transference to an electrode at 38° , and a p_{H} -time curve taken. A typical form is shown in Fig. 1, curve 3. The first part shows a decrease of p_{H} with temperature which corresponds to the change in p_{H} incident to the warming of the blood. This is complete in about 4–5 minutes, and is followed by a steady p_{H} period. The "first acid change," which is apparently delayed by the cooling, then follows. The "plateau" lasts about 5 minutes, and has been shown to agree with the p_{H} of a sample of the same blood measured immediately after it was shed. In one experiment the blood was left for as long as 17 hours on ice before measurement. This last method is in our opinion the best, when the p_{H} cannot be measured at 38° immediately. It does not require the introduction of any correction, and can be done at any convenient time subsequent to the puncture. The addition of oxalate and fluoride is not essential.

SUMMARY.

1. An increase in the hydrogen ion concentration of blood has been found to take place soon after the blood is shed.
2. This change has a mean value of $-0.05 p_{\text{H}}$ at 38° and at 28° . It takes place within about 6 minutes at 38° , and 11 minutes at 28° . It does not occur until after 1.5–2 hours at room temperature.
3. The change is unaffected by sodium fluoride, anticoagulants, potassium cyanide or thymol. It takes place in laked blood and centrifuged laked blood but not in plasma.
4. It is suggested that blood should be cooled to 0° before subsequent p_{H} measurement at 38° . In this way the acid change can be delayed long enough to enable a satisfactory p_{H} estimation to be made.

We wish to thank Prof. J. Barcroft, Prof. Arthur Ellis, and Prof. C. Lovatt Evans for much helpful interest and criticism, Miss N. Henderson for assistance in the early part of the work, the Medical Research Council and the London Hospital Medical College Research Fund for defraying the expenses of the research, and the Cambridge Instrument Co. for the loan of instruments.

REFERENCES.

- Evans (1922). *J. Physiol.* **56**, 146.
Friedemann, Cotonio and Shaffer (1927). *J. Biol. Chem.* **73**, 335.
Harington and Van Slyke (1924). *J. Biol. Chem.* **61**, 575.
Hirsch (1924). *J. Biol. Chem.* **61**, 795.
Hughes (1928). *J. Chem. Soc.* 491.
Kerridge (1926). *J. Sci. Inst.* **3**, 404.
Kugelmass (1922). *Compt. Rend. Soc. Biol.* **87**, 802.
Martin and Lepper (1926). *Biochem. J.* **20**, 1071.
Ross (1921). *J. Physiol.* **54**, 80.
Van Slyke, Hastings and Neill (1922). *J. Biol. Chem.* **54**, 507.